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Synthetic Control of Transcription: From Hybrid Promoters to Promoter Engineering to Synthetic Operon Design

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Synthetic control of transcription:
From hybrid promoters to promoter engineering to synthetic operon design

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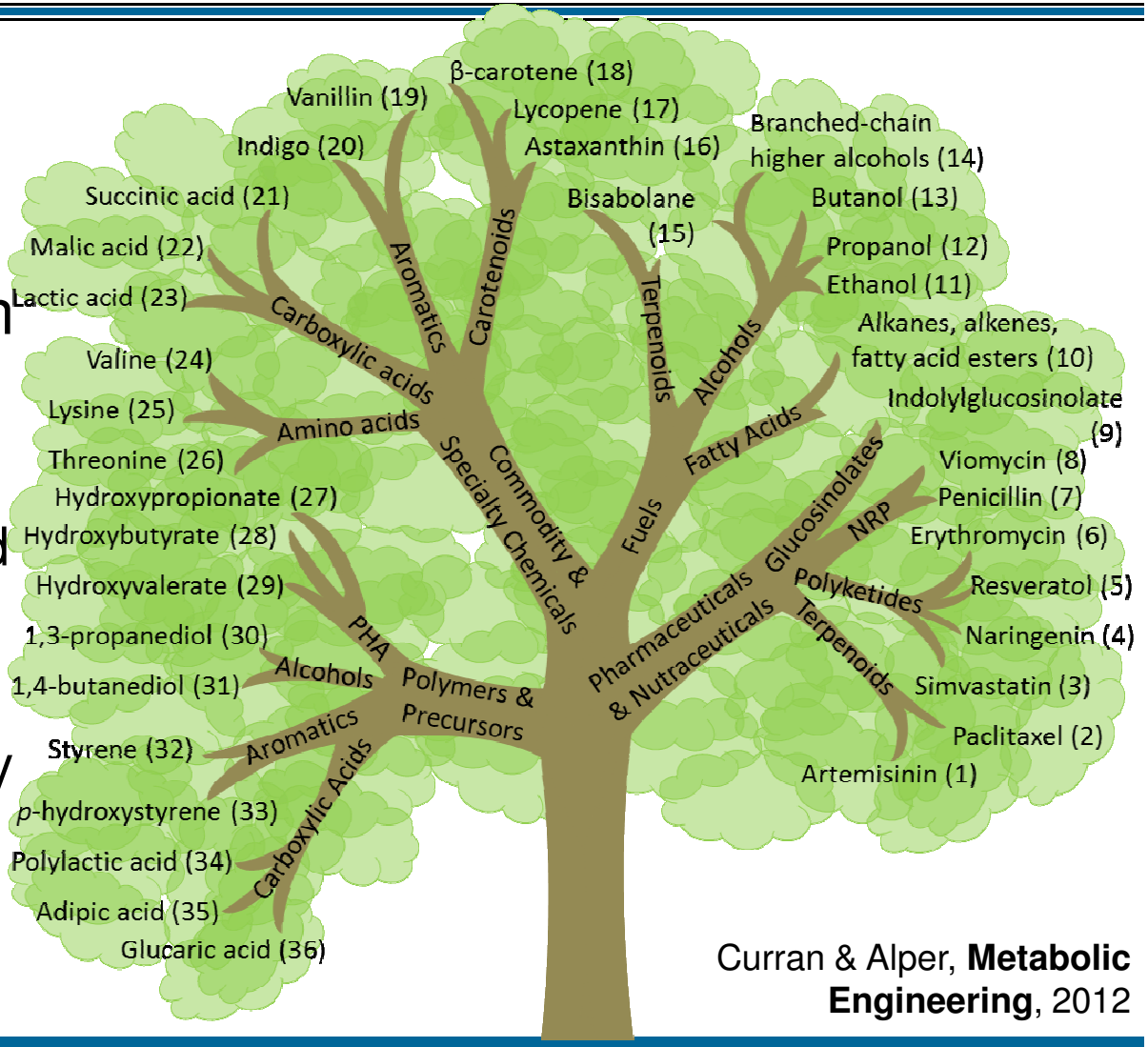
Metabolic Engineering has opened up possibilities…

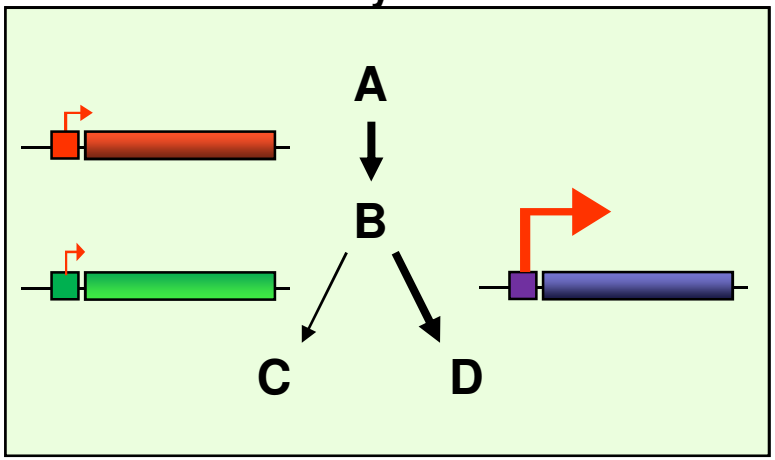
- Metabolic engineering advances have expanded the breath of chemicals produced by cells
  - Pharmaceuticals and Nutraceuticals
  - Fuels
  - Commodity/Specialty Chemicals
  - Polymers and Precursors



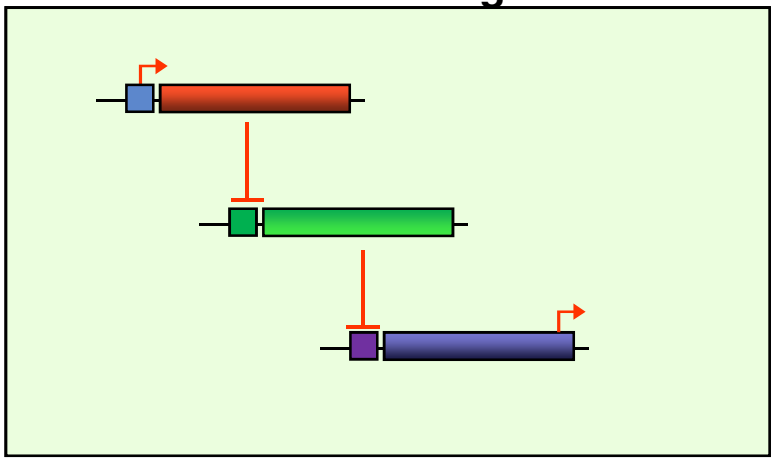
Curran & Alper, **Metabolic Engineering**, 2012

…but, requires synthetic control elements...

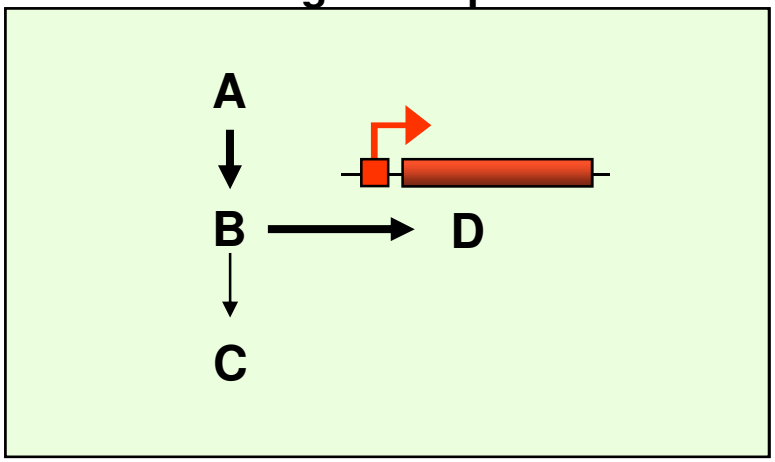
Pathway Control



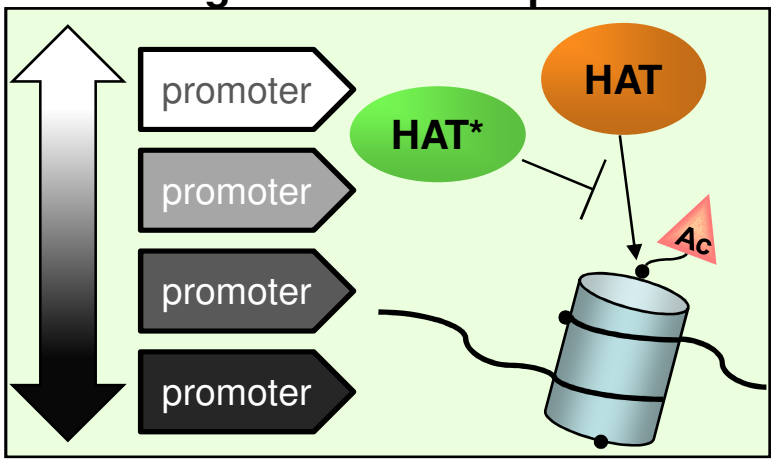
Circuit Design



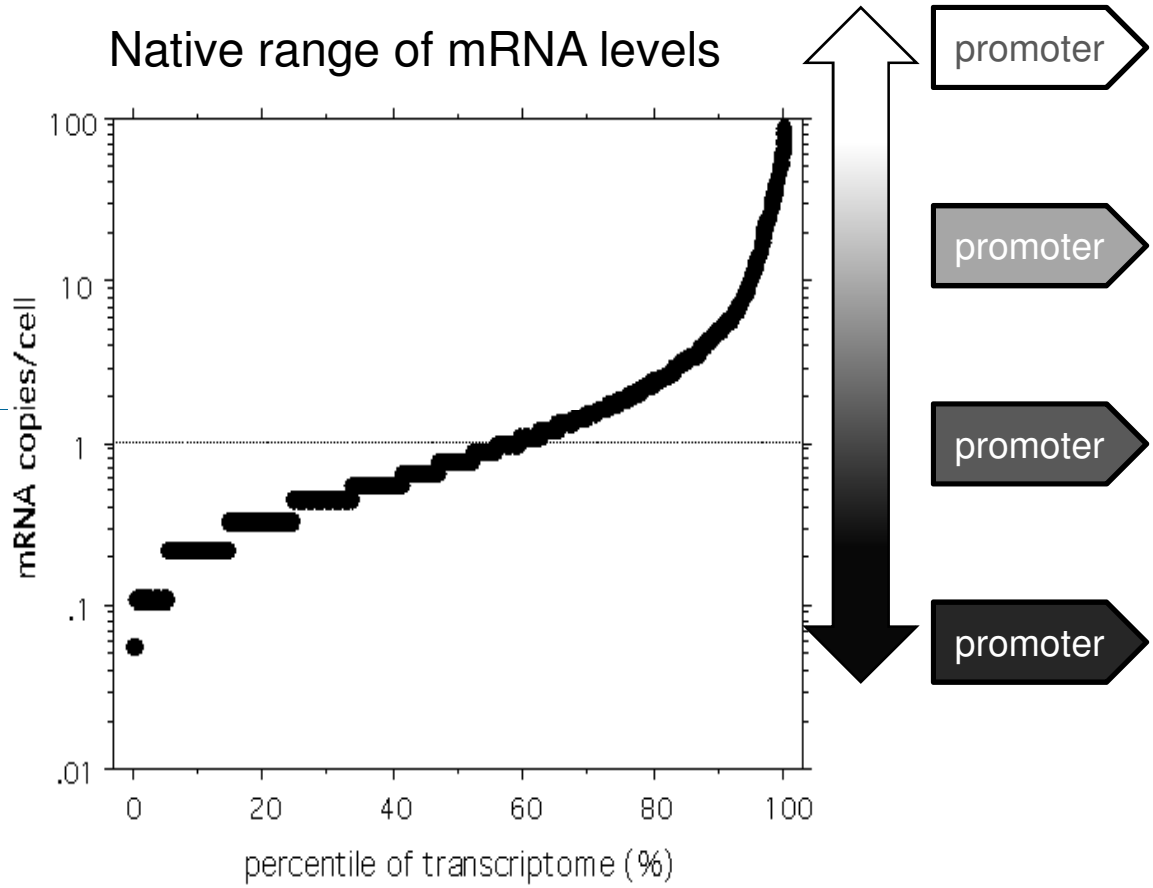
Heterologous Expression



Probing via Graded Expression



…with a range of expression capacities.

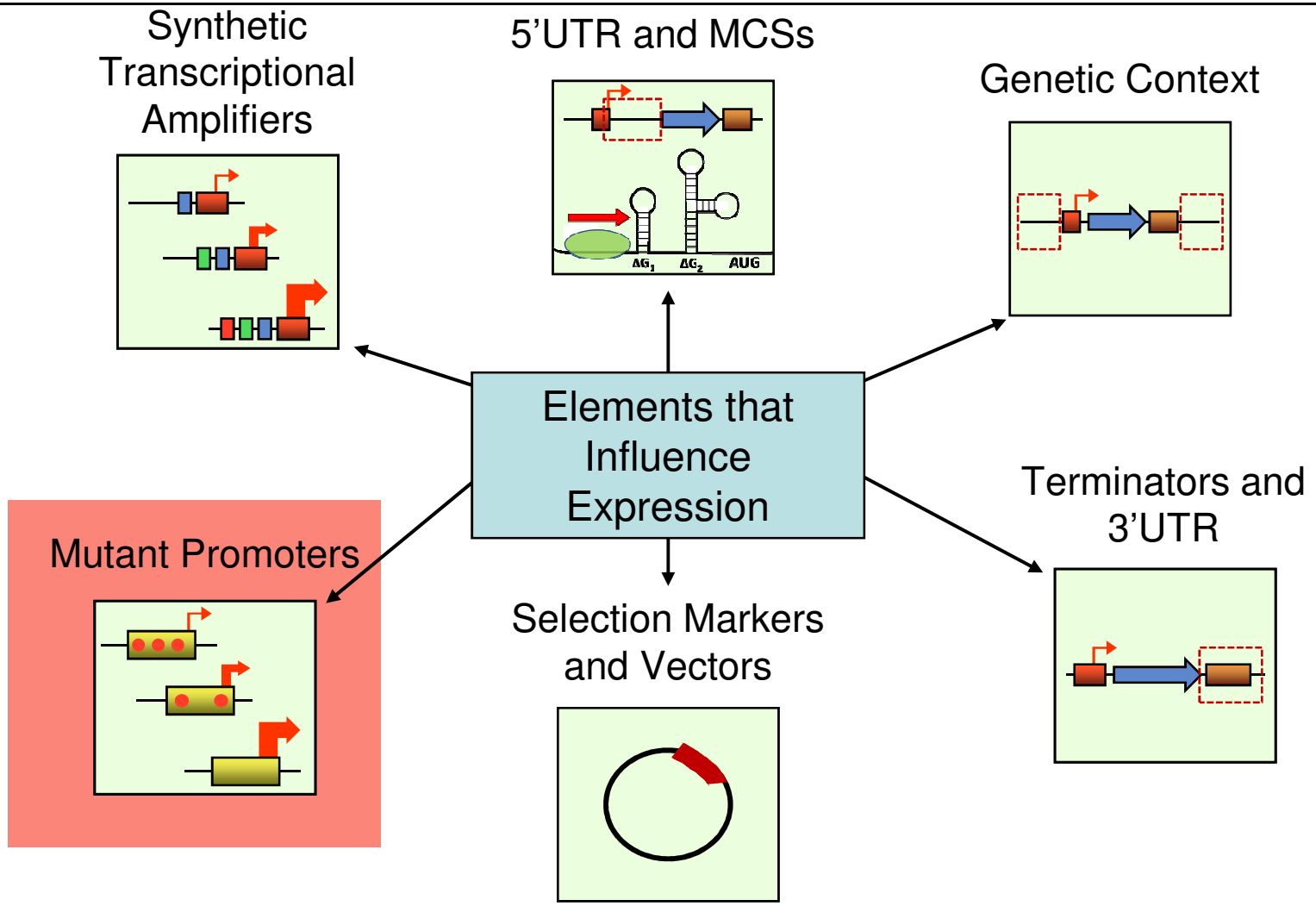


Require a promoter series(s) that span above the highest native expression down to essentially zero expression:

Affords both amplification and knockdowns.

Holstege, et al. (1998), Cell 95:717-728.

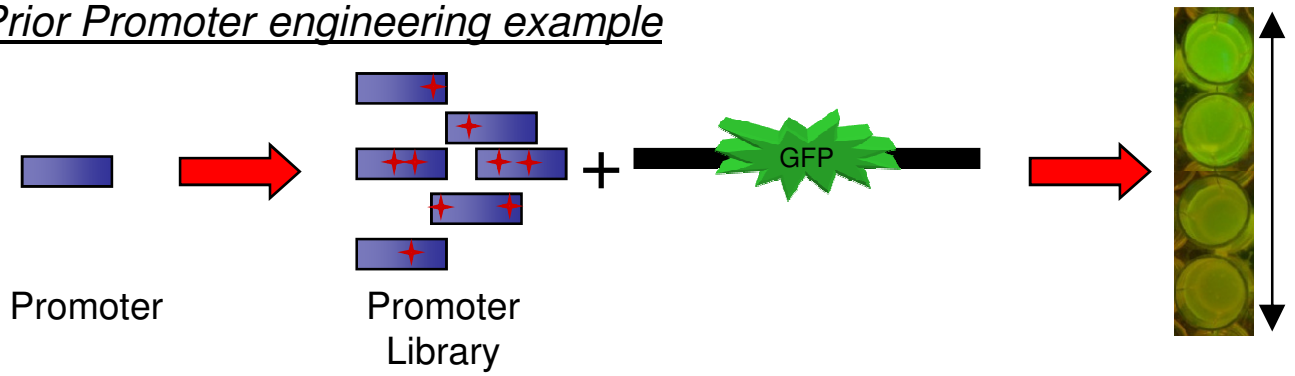
Synthetic control of transcription occurs at many levels



Designing novel genetic control elements

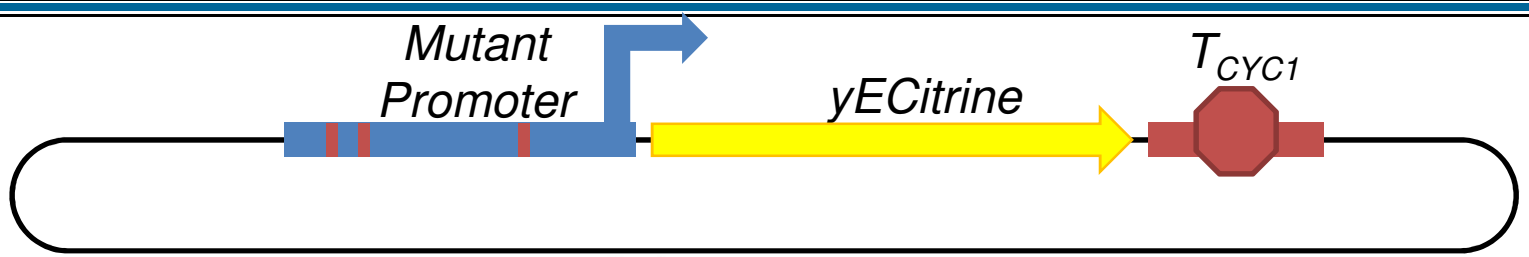
- Promoter Engineering is an effective method for generating a collection of genetic control elements

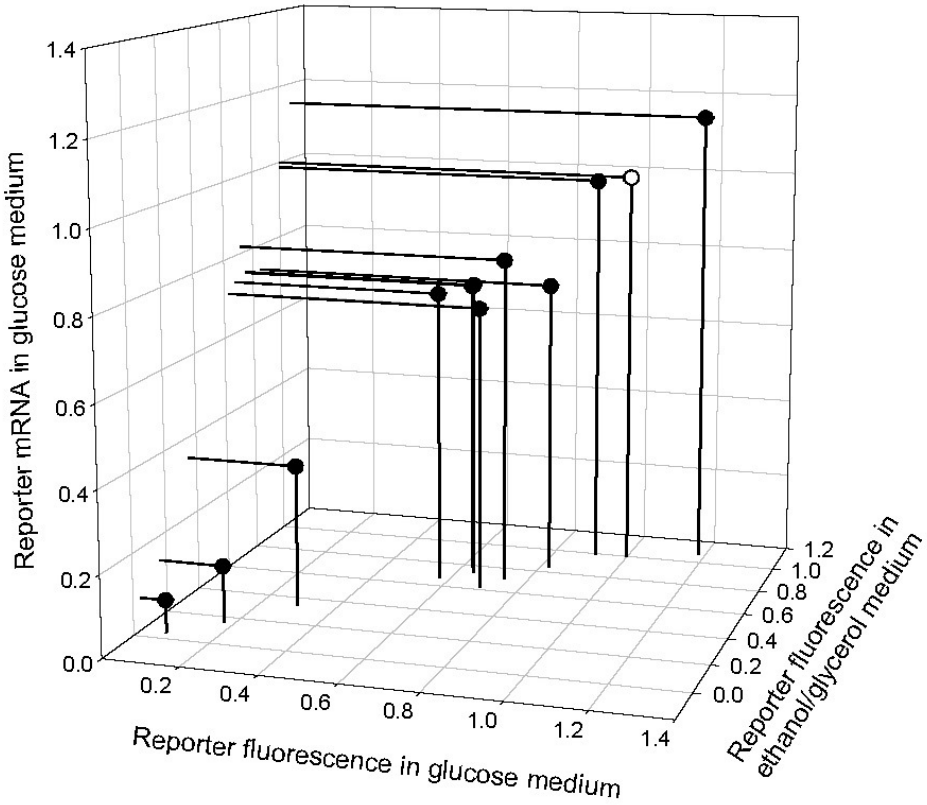
Prior Promoter engineering example



Alper et al. ***PNAS***, 2005.

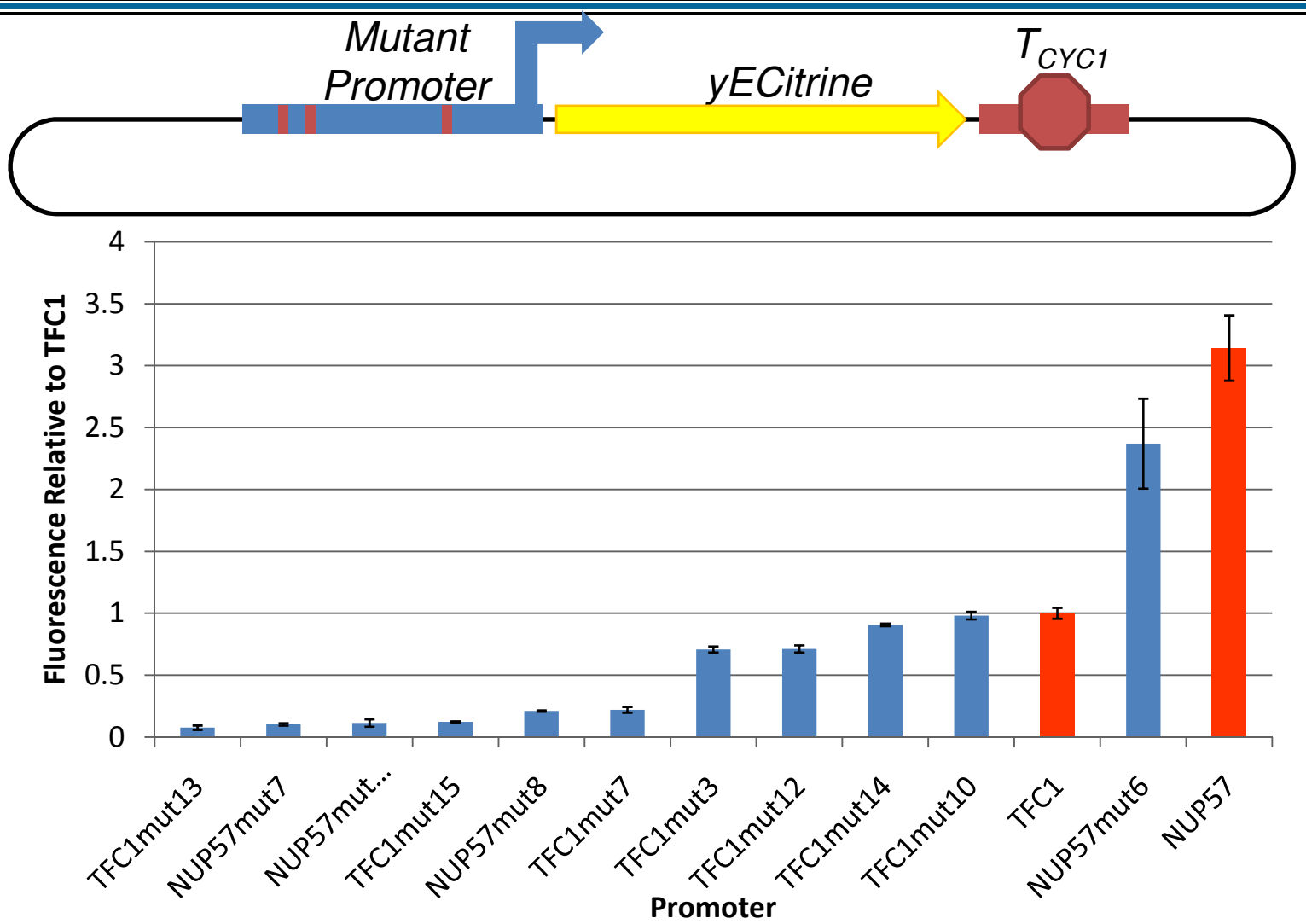
Development of diversified promoters





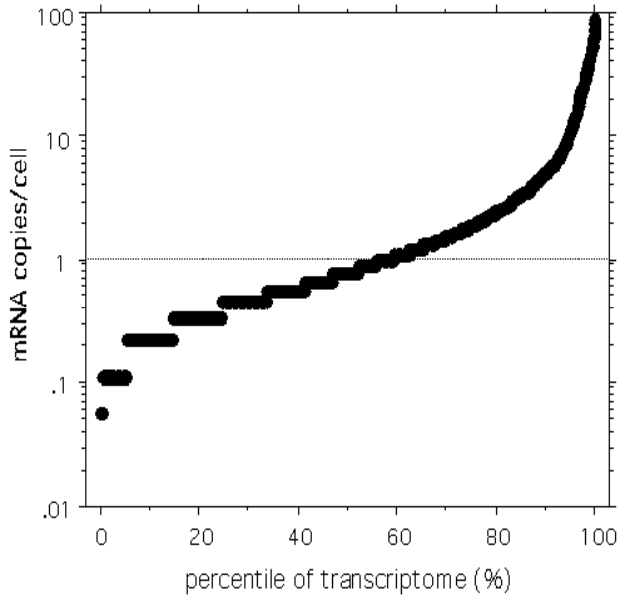
Nevoigt et al. ***AEM***, 2006.

Development of novel low-strength promoters

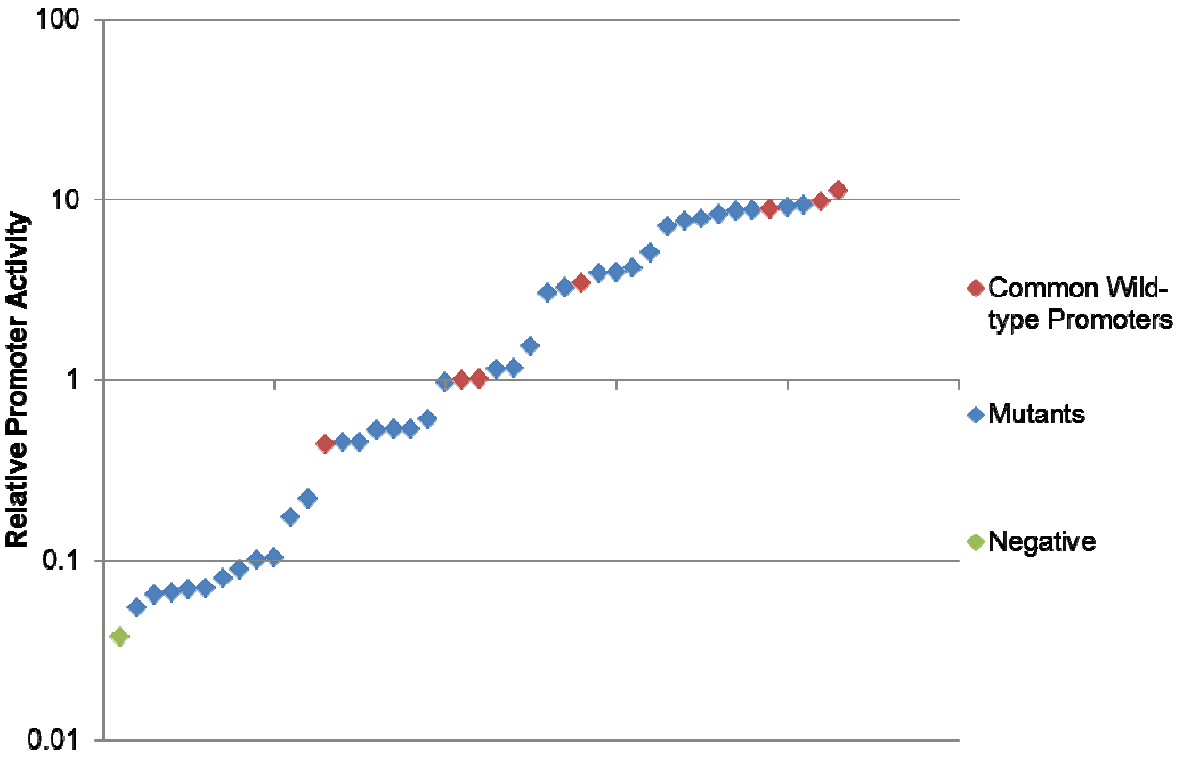


An expanding range of yeast promoters

Native range of mRNA levels



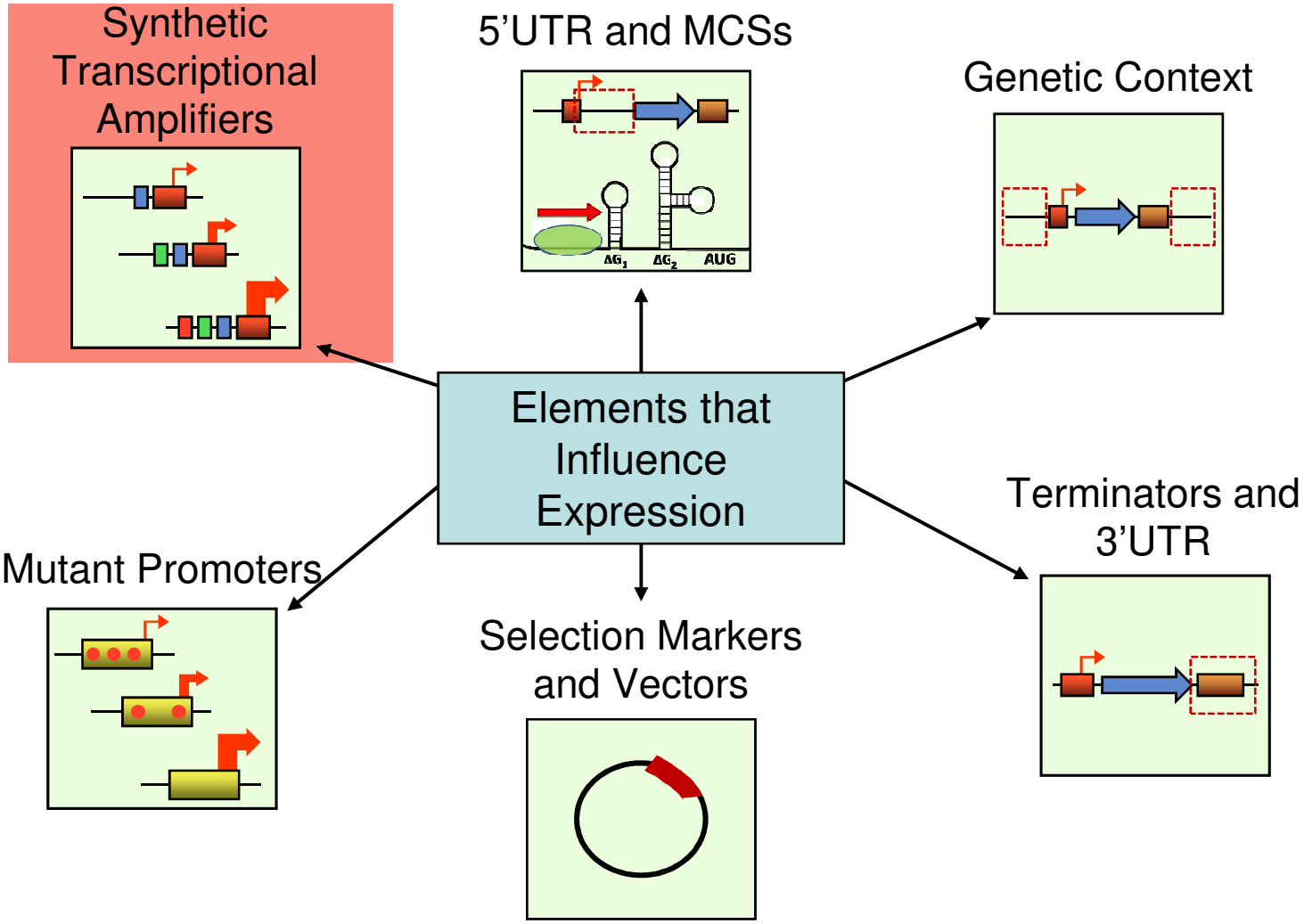
Holstege, et al. (1998), Cell 95:717-728.



Mutant Promoter Libraries

- Promoter engineering via random mutagenesis is effective at creating diversity
- Bias in promoter libraries toward **lower expression capacity**
- Expression range nearly matches “native expression levels”—need more sequence diversity
- Require novel tools for increasing strength of promoters

Synthetic control of transcription occurs at many levels



Promoter Engineering in *Yarrowia lipolytica*

Yarrowia lipolytica is a fully sequenced, oleaginous yeast

Advantages

- Naturally accumulates fatty acids (including linoleic acid) on food source with high C:N ratio
- Semi-developed genetic tools
- Thrives on non-conventional carbon sources

Challenges

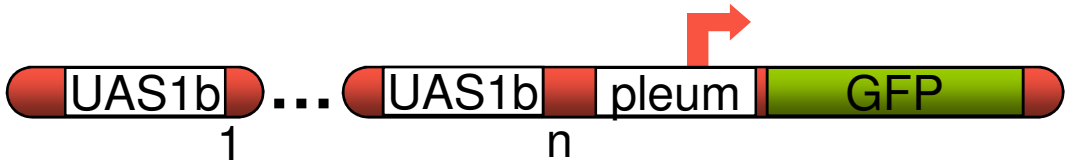
- Issues with immature genetic tools and expression levels
  - Low plasmid copy number
  - **Lack of genetic tools**
  - **"Strong" promoters do not readily exist**

Y. lipolytica Promoter Engineering via tandem UAS sequences

Prior research suggested linking upstream activating sequences (UAS) to a minimal promoter can create function. Analyzed 1 to 4 UAS site.

Madzak et al., *Microbiology*, 1999

We sought to evaluate potential of modulating promoter activity by tandem copies of UAS1b sites (n = 1 to 32)

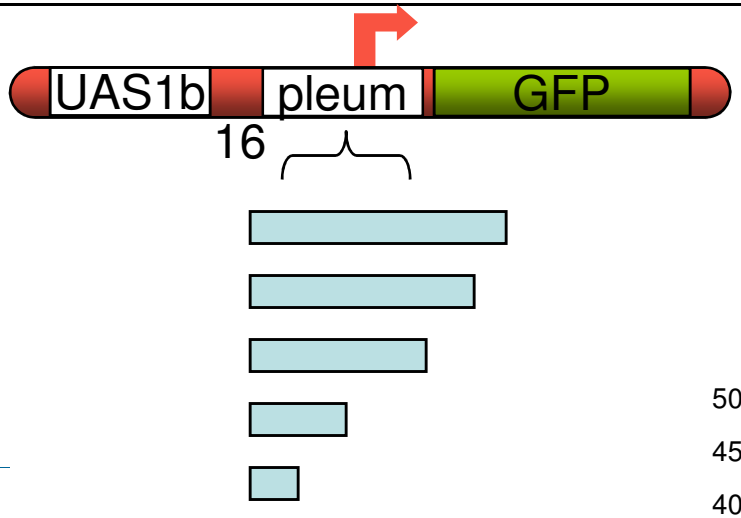


Creation of a strong promoter set in *Y. lipolytica*

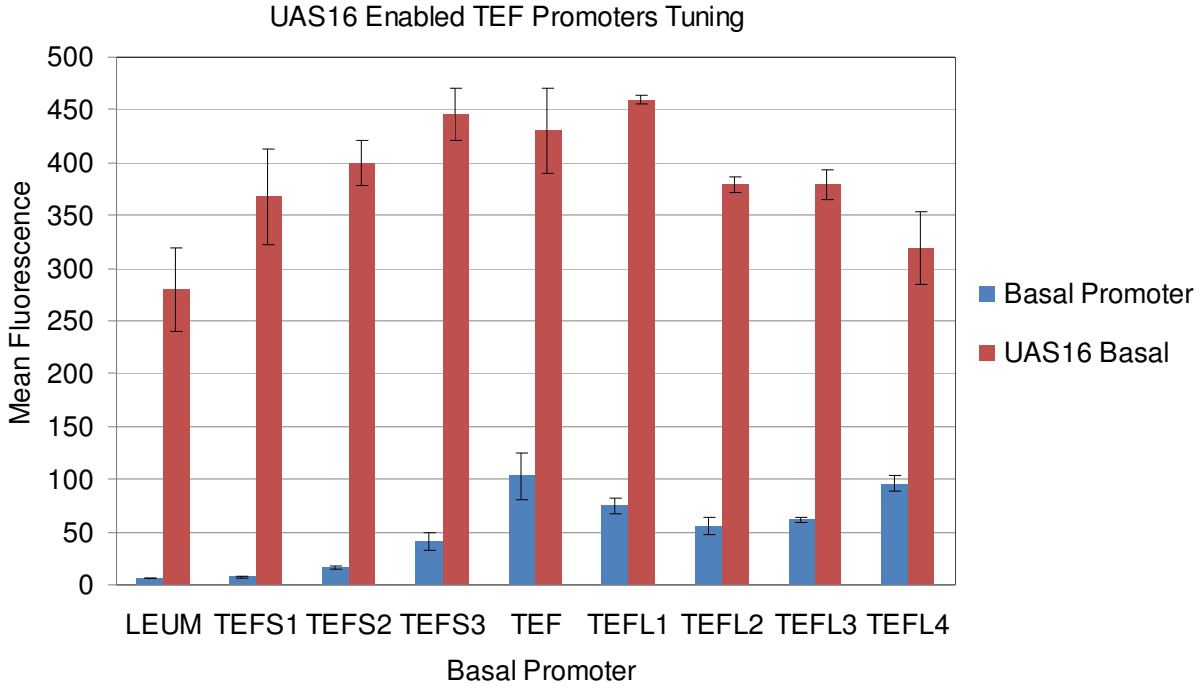
Promoter Construct

Blazeck et al., **AEM**, 77(22), 2011

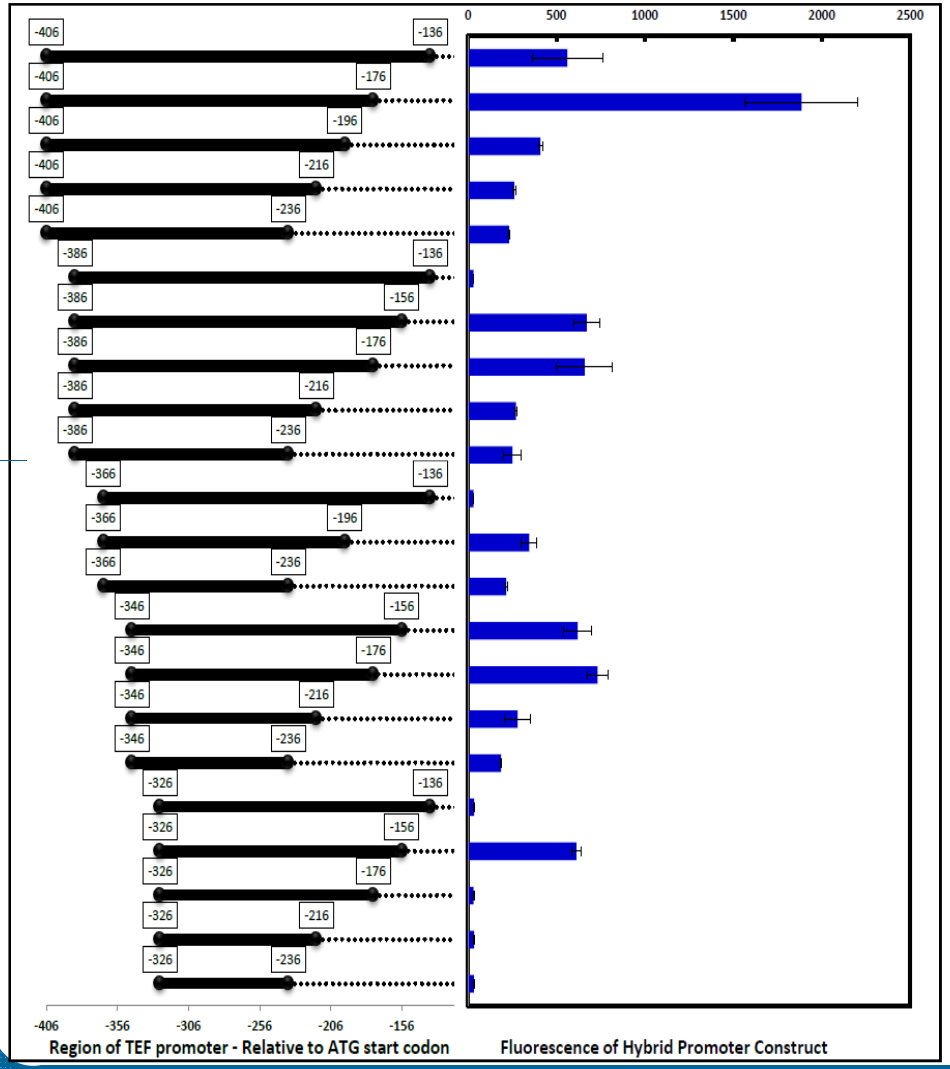
Extension of hybrid promoter strategy

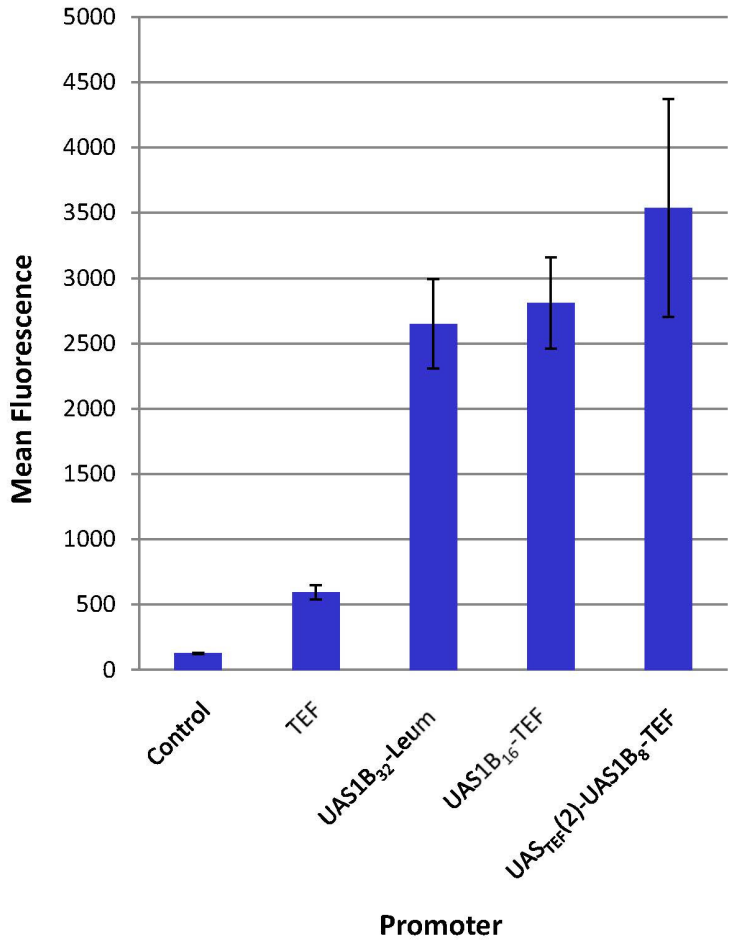


TEF promoter truncation series



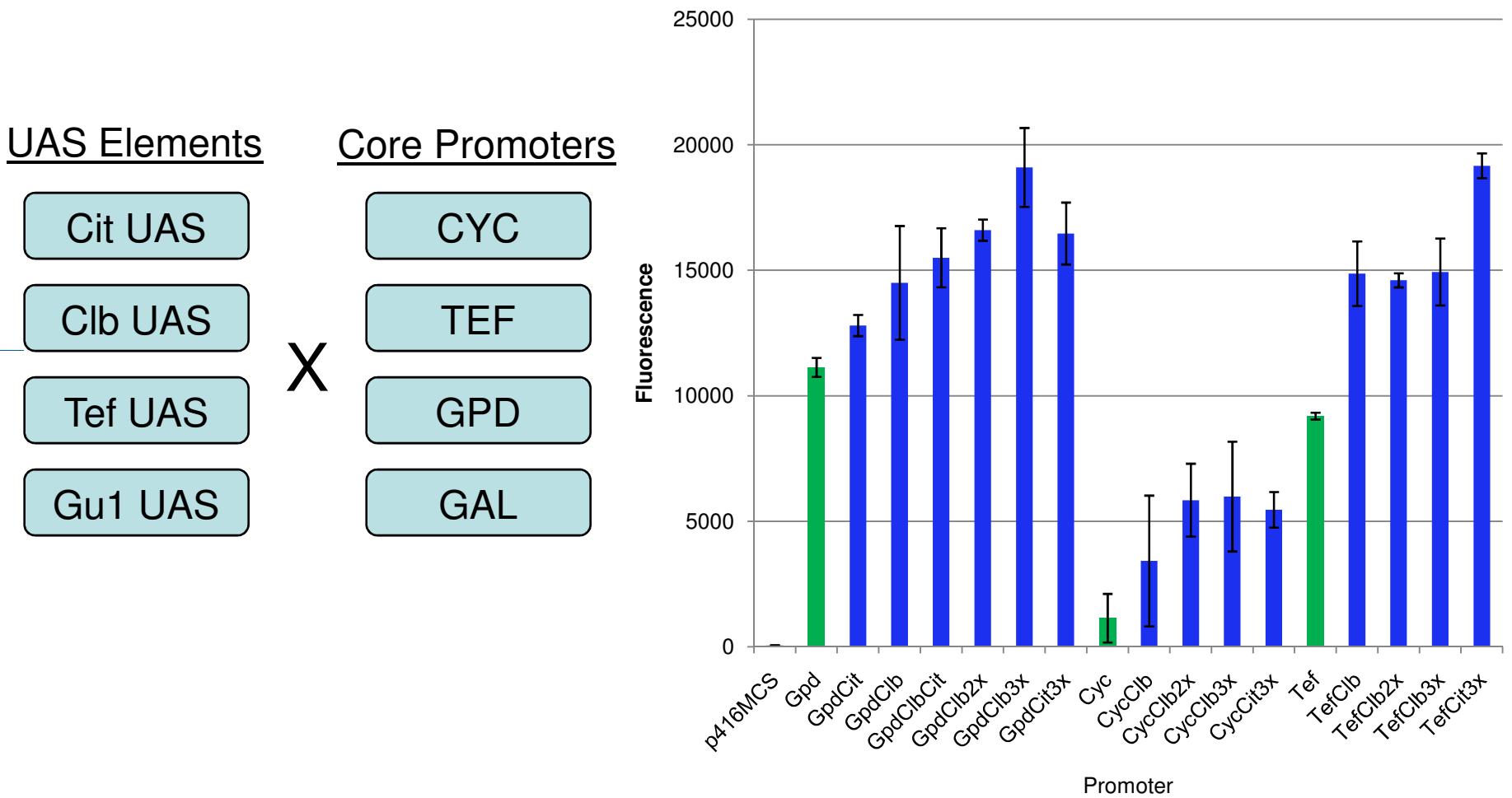
Identification and use of a TEF UAS





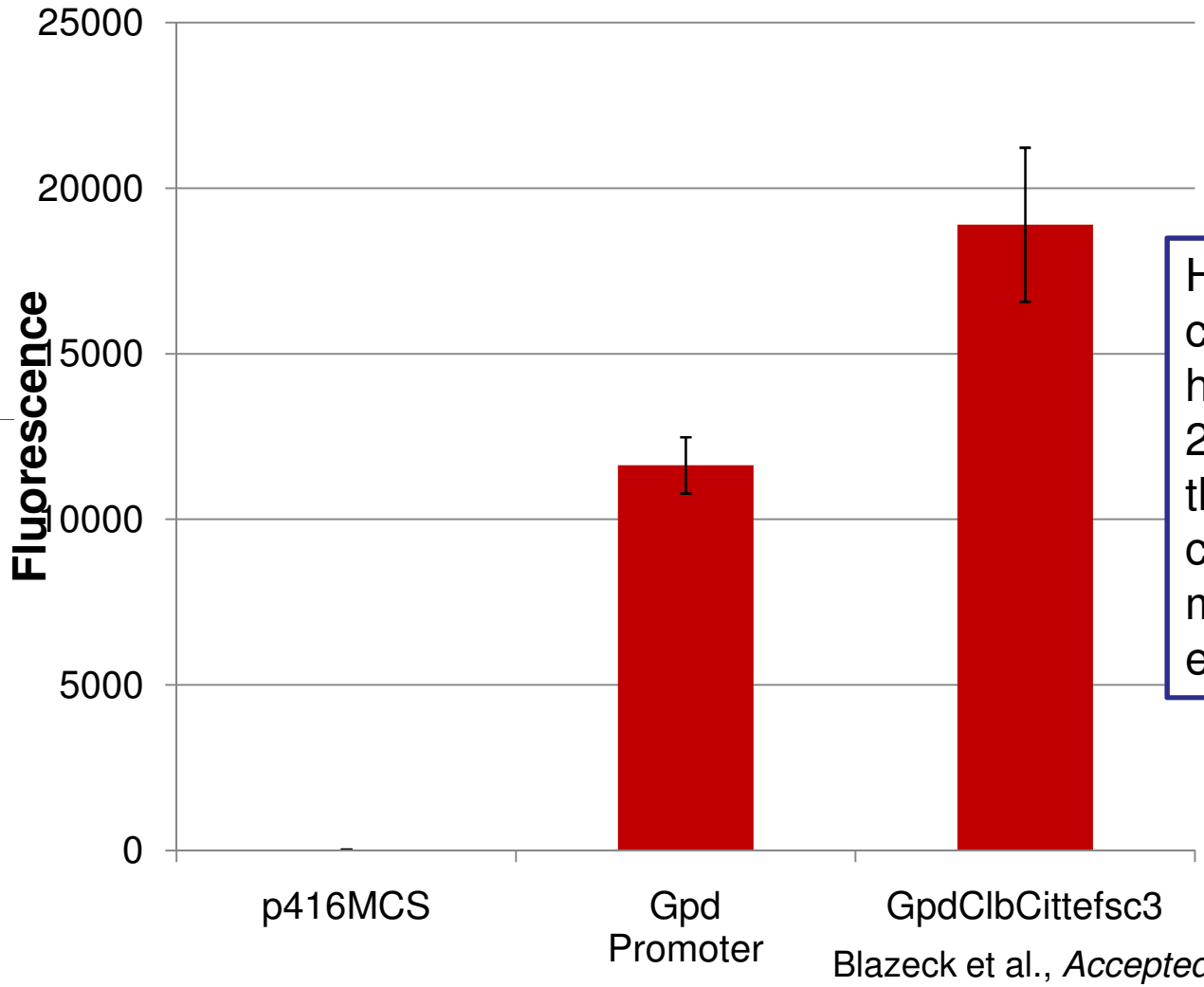
Blazeck et al., Submitted, **AMB**, 2012

Expanding the hybrid promoter approach to *S. cerevisiae*



Blazeck et al., *Accepted/In Press*, **Biotech Bioeng**, 2012

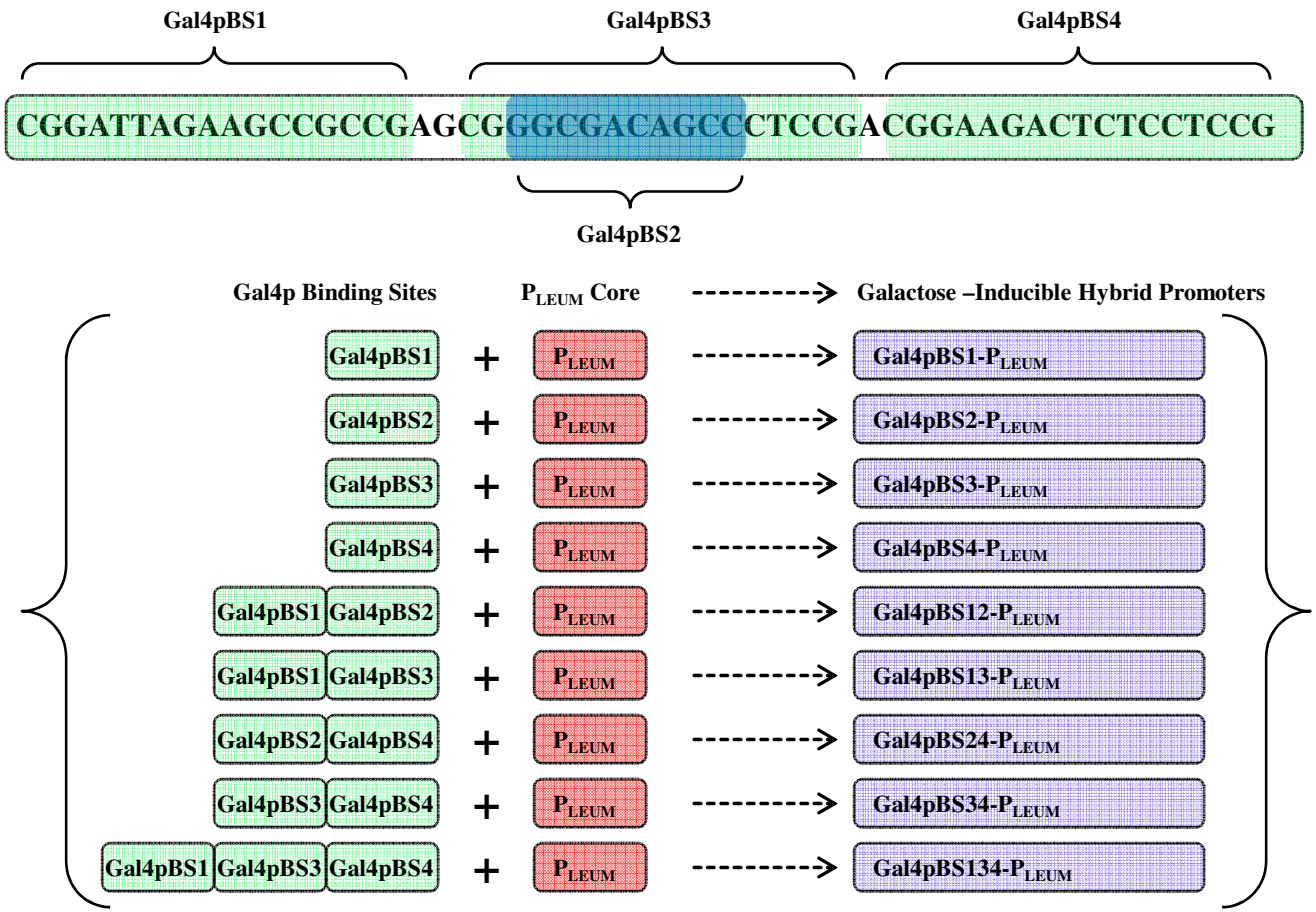
Creating the strongest constitutive promoter



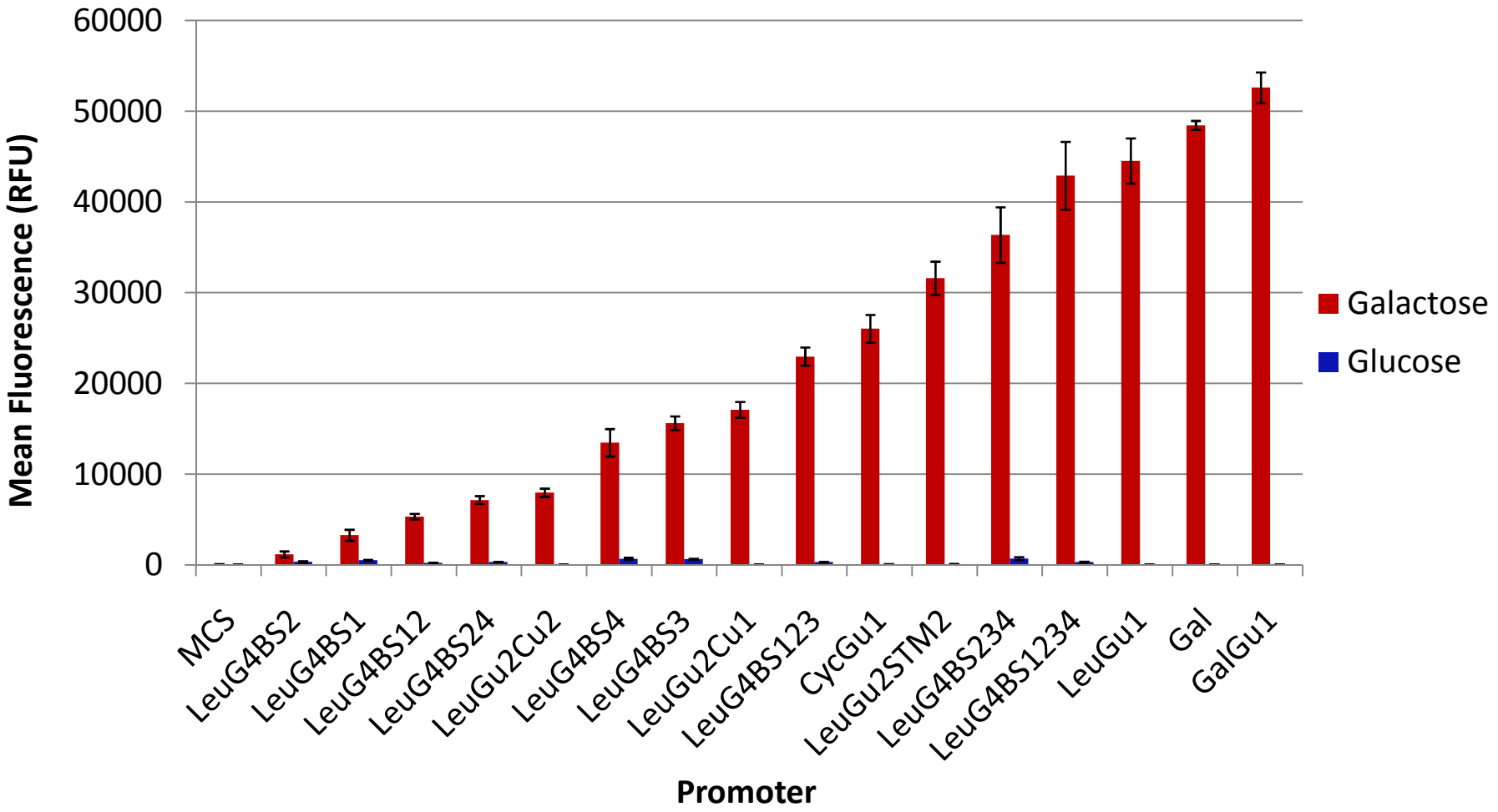
Hybrid promoter approach created a promoter 60% higher on fluorescence and 2.5 fold higher in mRNA than the strongest constitutive promoter by mixing disparate UAS elements

Blazeck et al., *Accepted/In Press*, **Biotech Bioeng**, 2012

Creating a range of inducible hybrid promoters



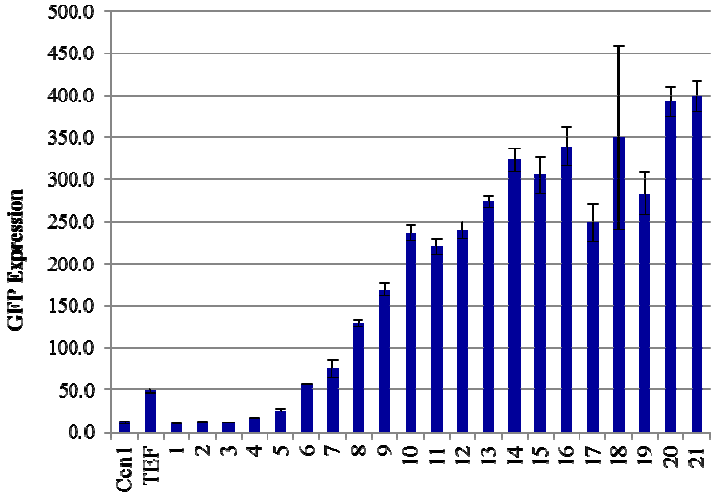
Creating a range of inducible hybrid promoters



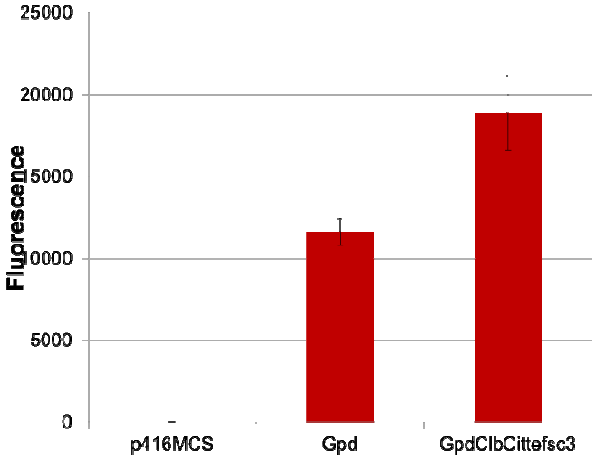
Blazeck et al., *Accepted/In Press*, **Biotech Bioeng**, 2012

Hybrid Promoter Engineering Examples

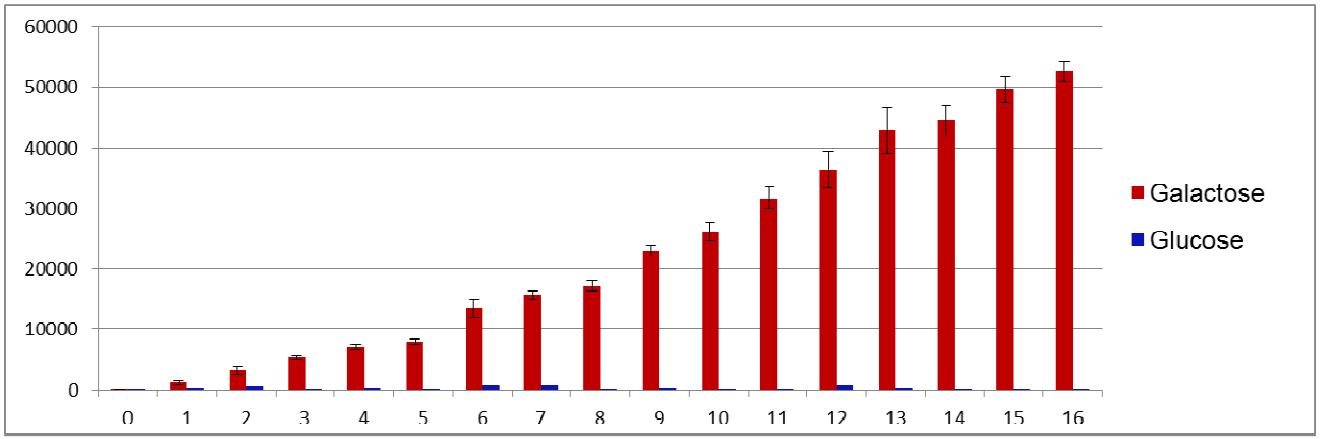
Largest library of promoters in *Y. lipolytica*



Strongest constitutive promoter in *S. cerevisiae*



Largest range of synthetic inducible promoters in *S. cerevisiae*

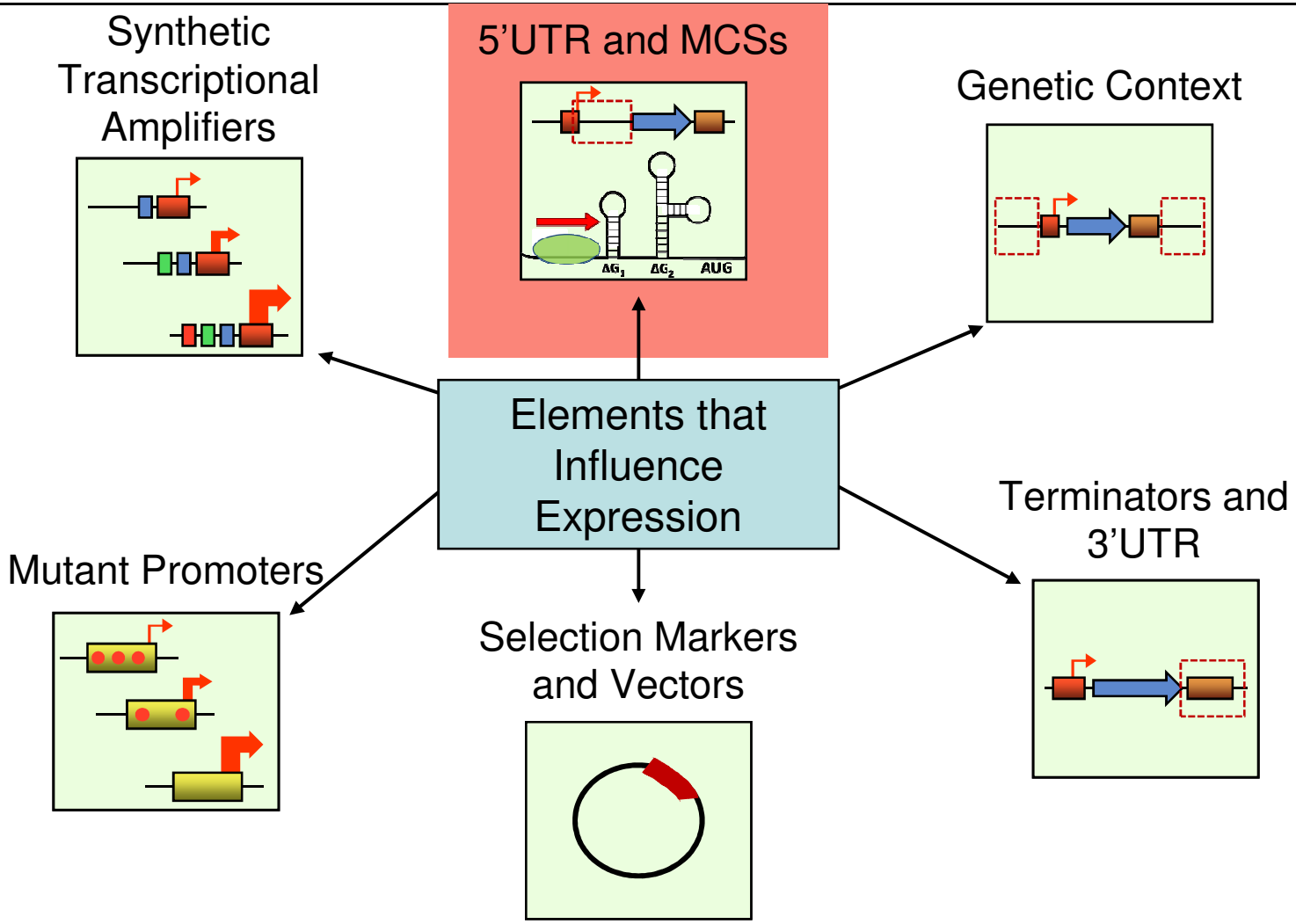


Blazeck et al., **AEM**, 77(22), 2011 & Blazeck et al., *Accepted/In Press*, **Biotech Bioeng**, 2012

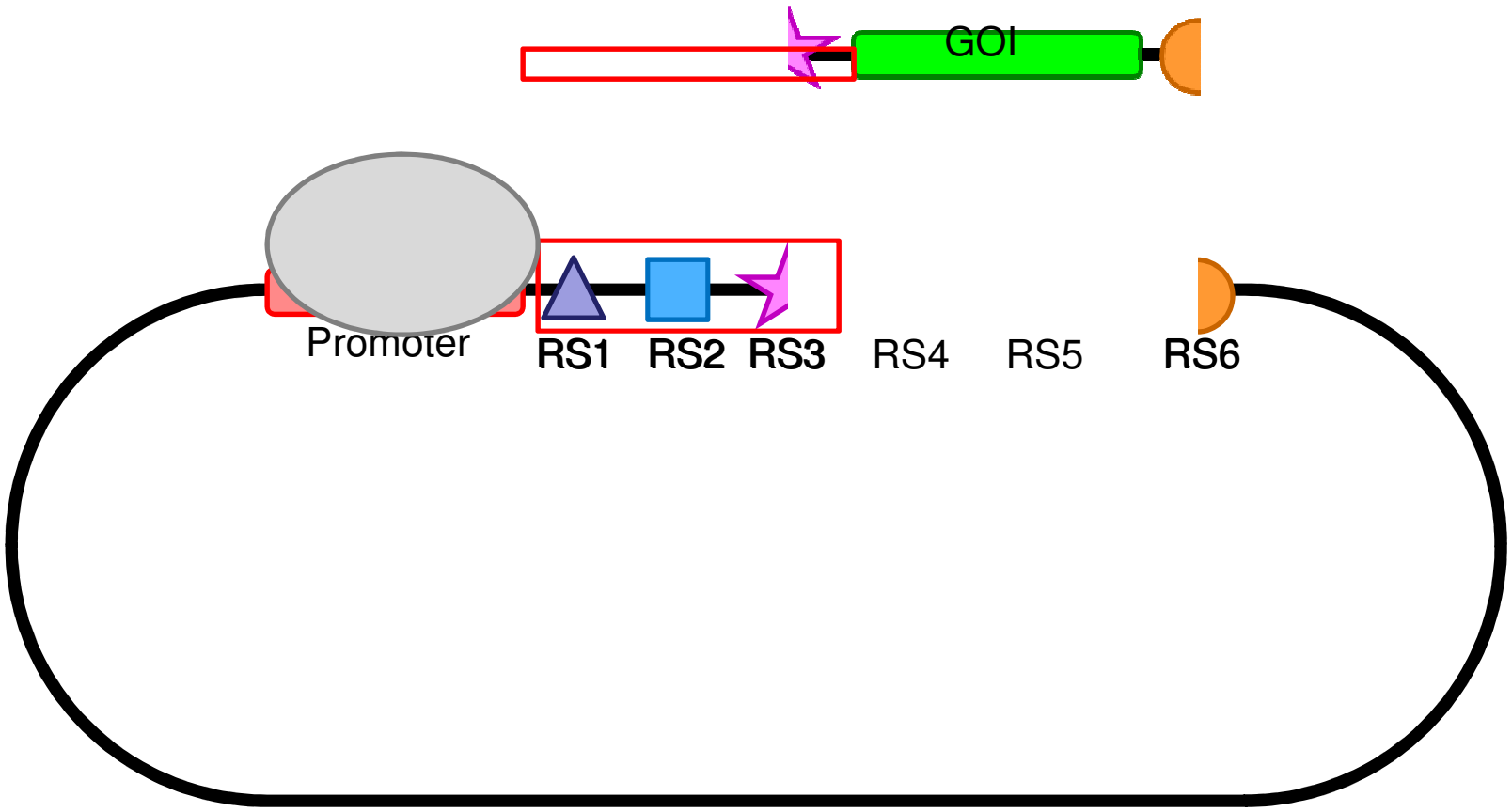
Synthetic, hybrid promoters

- UAS elements serve as synthetic transcriptional amplifiers
- UAS elements and core promoters can serve as modular synthetic components
- Hybrid promoter engineering can amplify expression and create highly-functional libraries

Synthetic control of transcription occurs at many levels



Multicloning sites influence 5'UTR sequences



Crook et al., *Nucleic Acids Research*, 39(14), e92, 2011

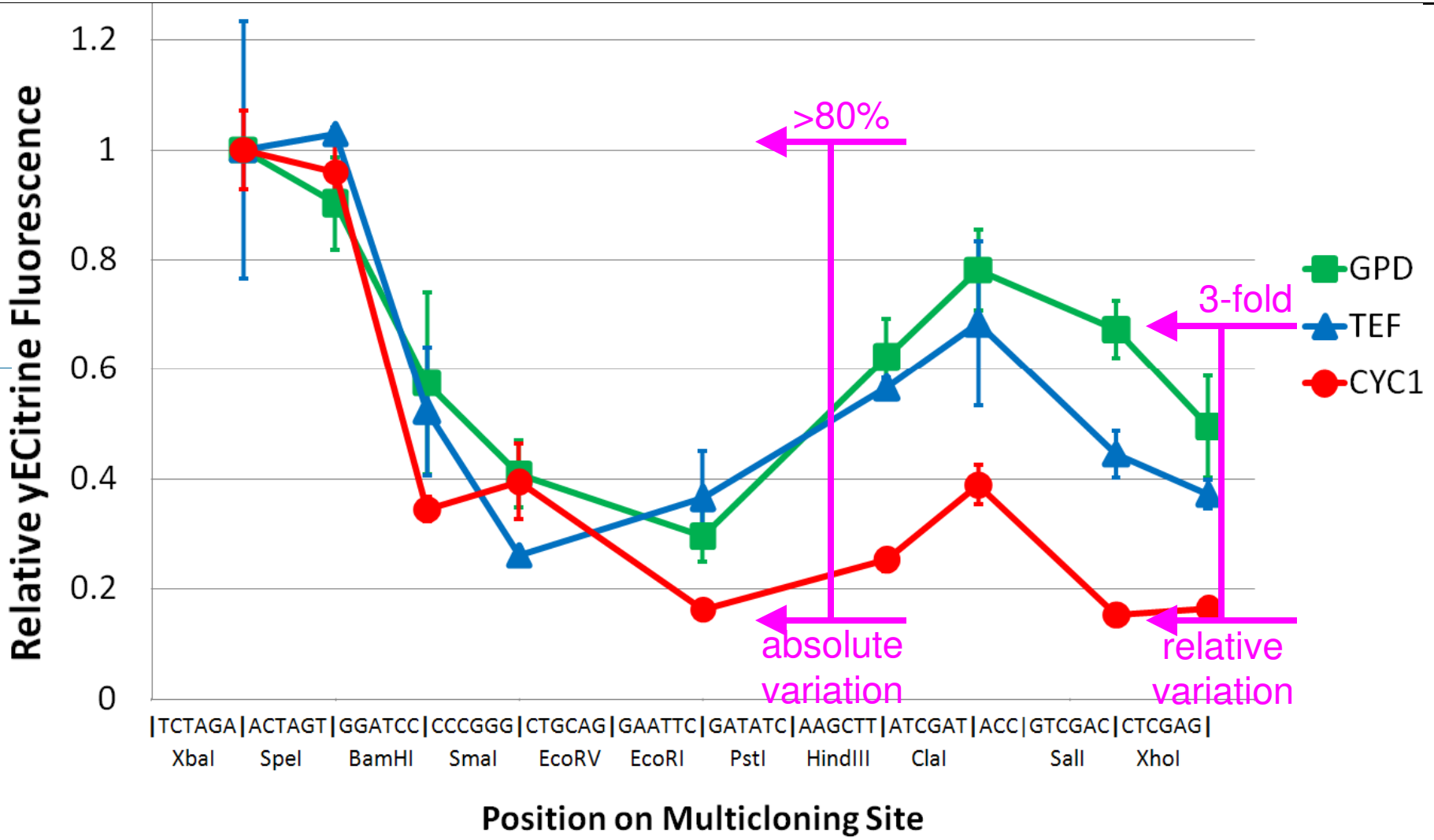
Model System

- Yeast model system *(S. cerevisiae)*
- pBluescript SK II Multicloning Site

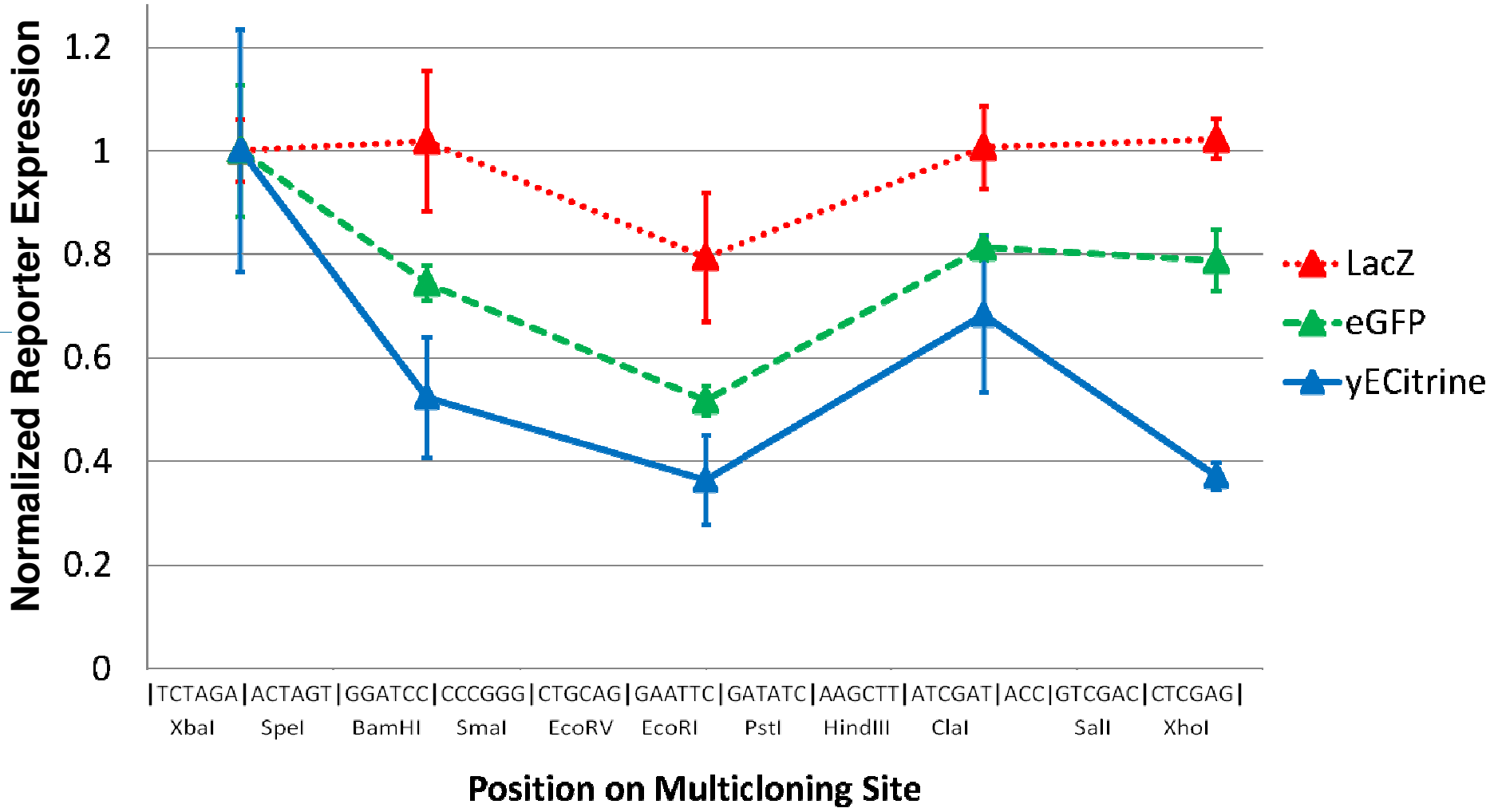
|TCTAGA|ACTAGT|GGATCC|CCCGGG|CTGCAG|GAATTC|GATATC|AAGCTT|ATCGAT|ACC|GTCGAC|CTCGAG|
XbaI SpeI BamHI SmaI EcoRV EcoRI PstI HindIII ClaI SalI XhoI

- CYC1, TEF, or GPD promoters
- yECitrine
  - Codon-optimized

Restriction site affects protein expression

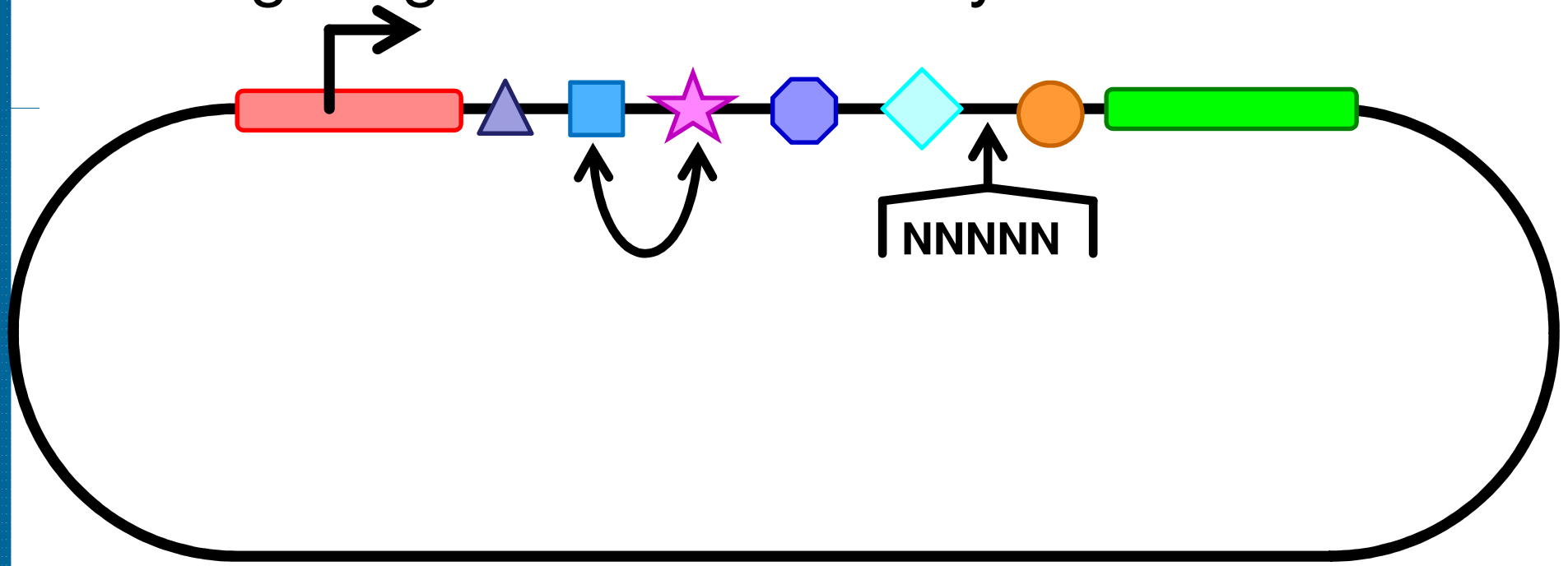


Effect is most pronounced with short, codon-optimized genes



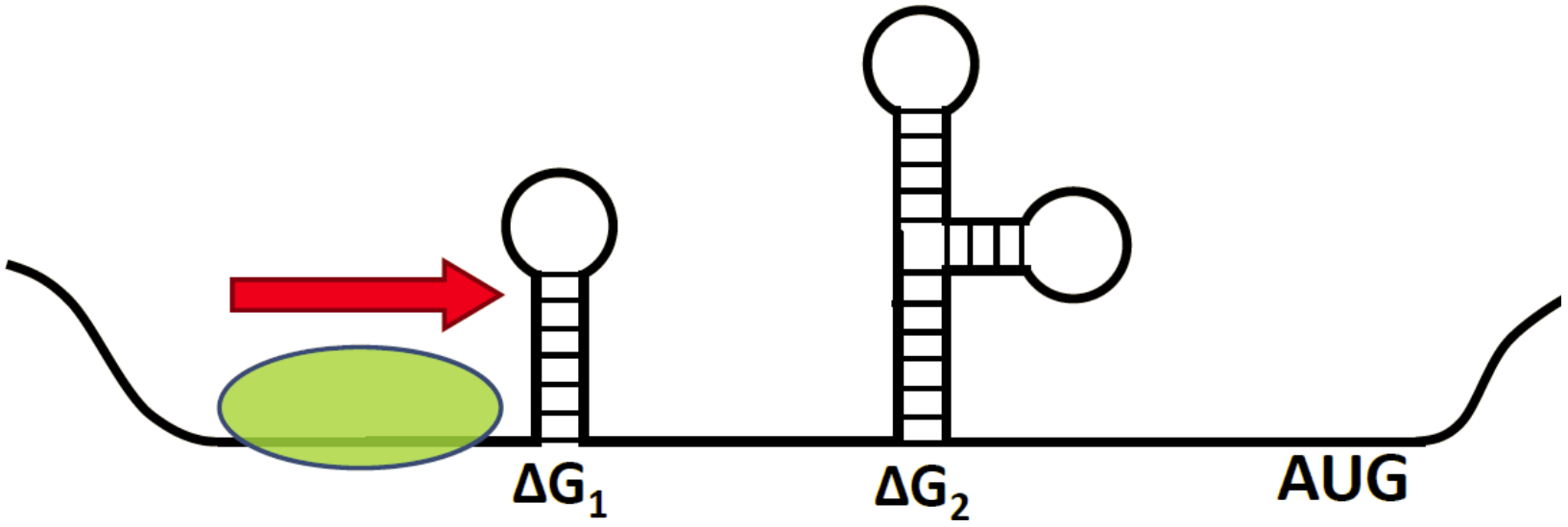
Modeling and re-designing MCSs

- Goal: Use model to re-design better MCSs not susceptible to position effect by mitigating 5'UTR secondary structure



Conceptual model of inhibition

- Region(s) of secondary structure in 5'UTR can impede ribosome progression



Modeling and re-designing MCSs: GPD/TDH3-based Promoter

TCTAGA	ACTAGT	GGATCC	CCCGGG	CTGCAG	GAATTC	GATATC	AAGCTT	ATCGAT	ACC	GTCGAC	CTCGAG
XbaI **1**	SpeI **2**	BamHI **3**	XmaI **4**	PstI **5**	EcoRI **6**	EcoRV **7**	HindIII **8**	ClaI **9**		SalI **10**	XhoI **11**

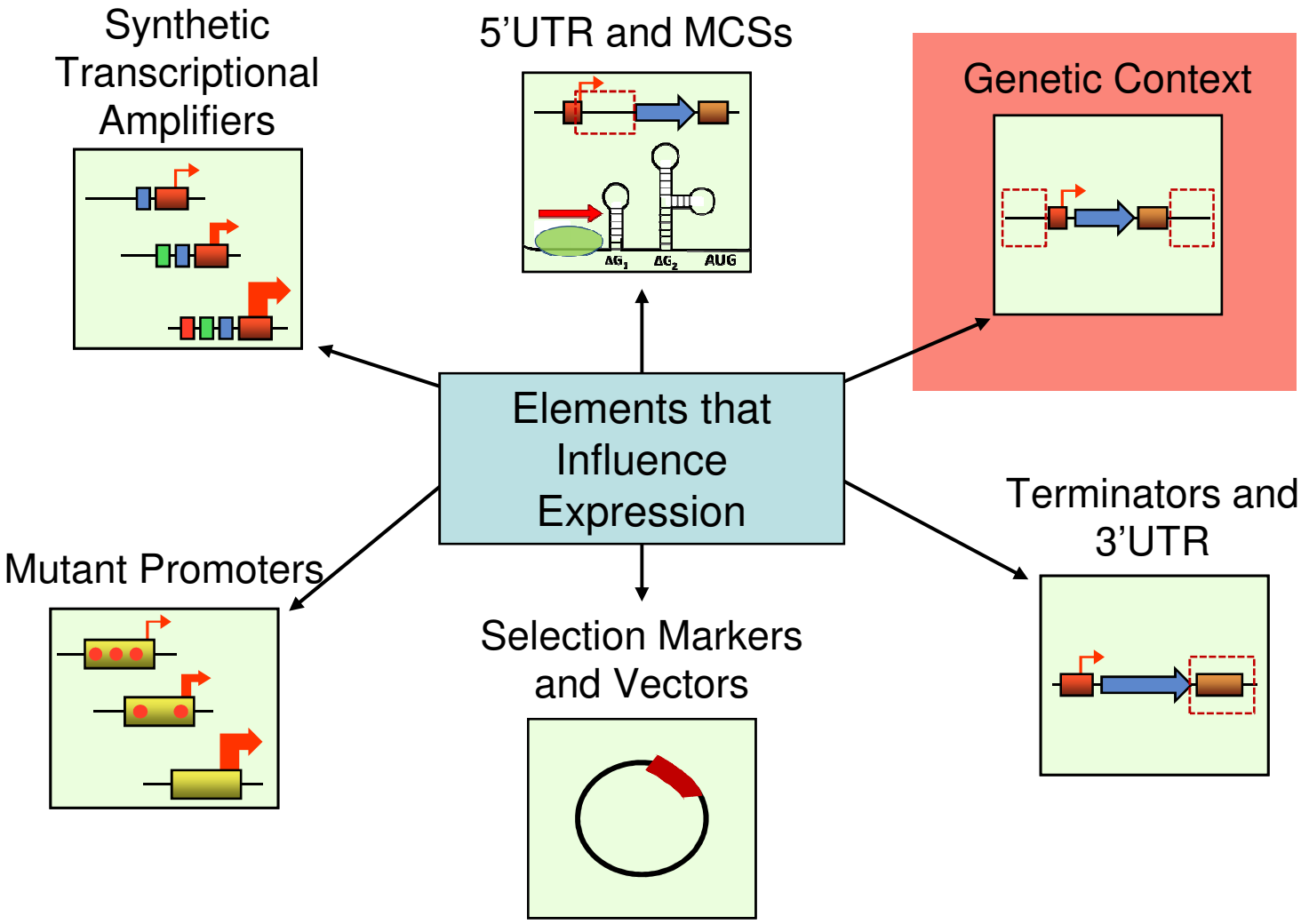
A	AAGCTT	ACTAGT	ATCGAT	AAA	GAATTC	TCTAGA	GGATCC	CTGCAG	CTCGAG	GTCGAC	CCCGGG	GATATC
	HindIII **1**	SpeI **2**	ClaI **3**		EcoRI **4**	XbaI **5**	BamHI **6**	PstI **7**	XhoI **8**	SalI **9**	XmaI **10**	EcoRV **11**

Crook et al., *Nucleic Acids Research*, 39(14), e92, 2011

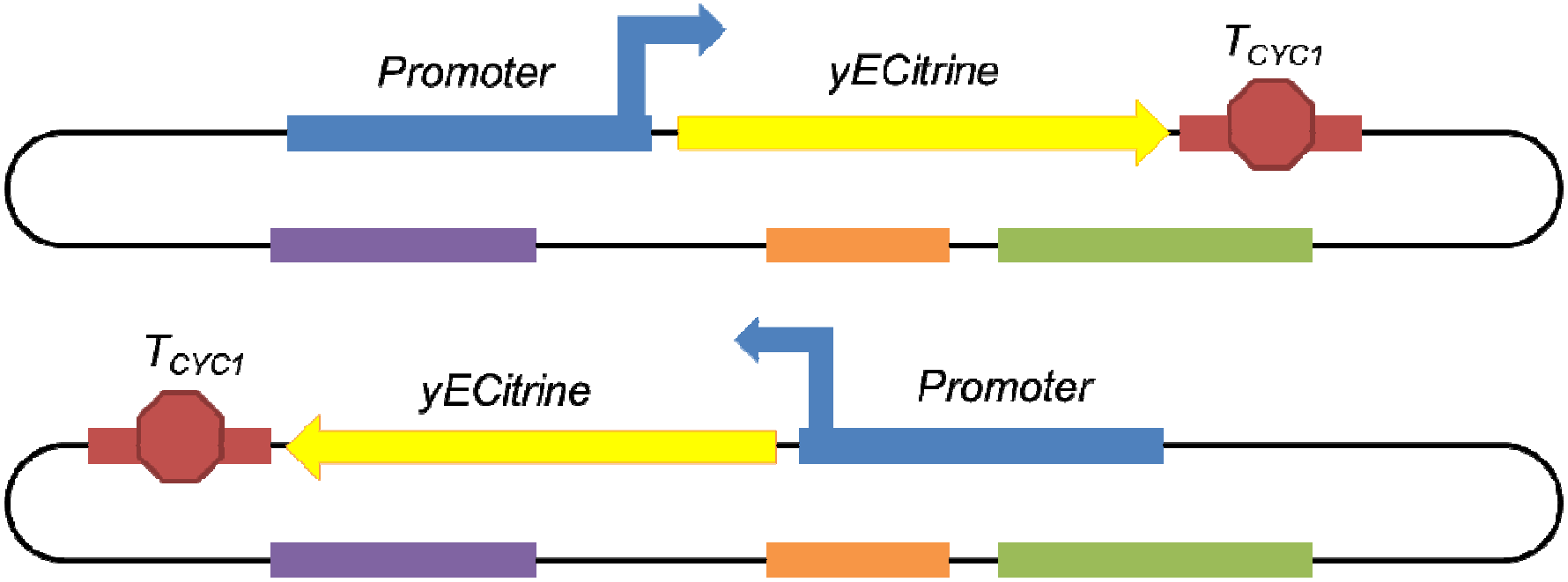
5'UTR Influence

- Choice of site in MCS can influence 5'UTR regions via secondary structure
- Most pronounced by short, codon-optimized genes (i.e. when transcription and translation rates are not limiting)
- Can use model-based approach to improve MCS design (esp. with GPD)
- Genetic context is important for characterizing parts

Synthetic control of transcription occurs at many levels



Importance of genetic context



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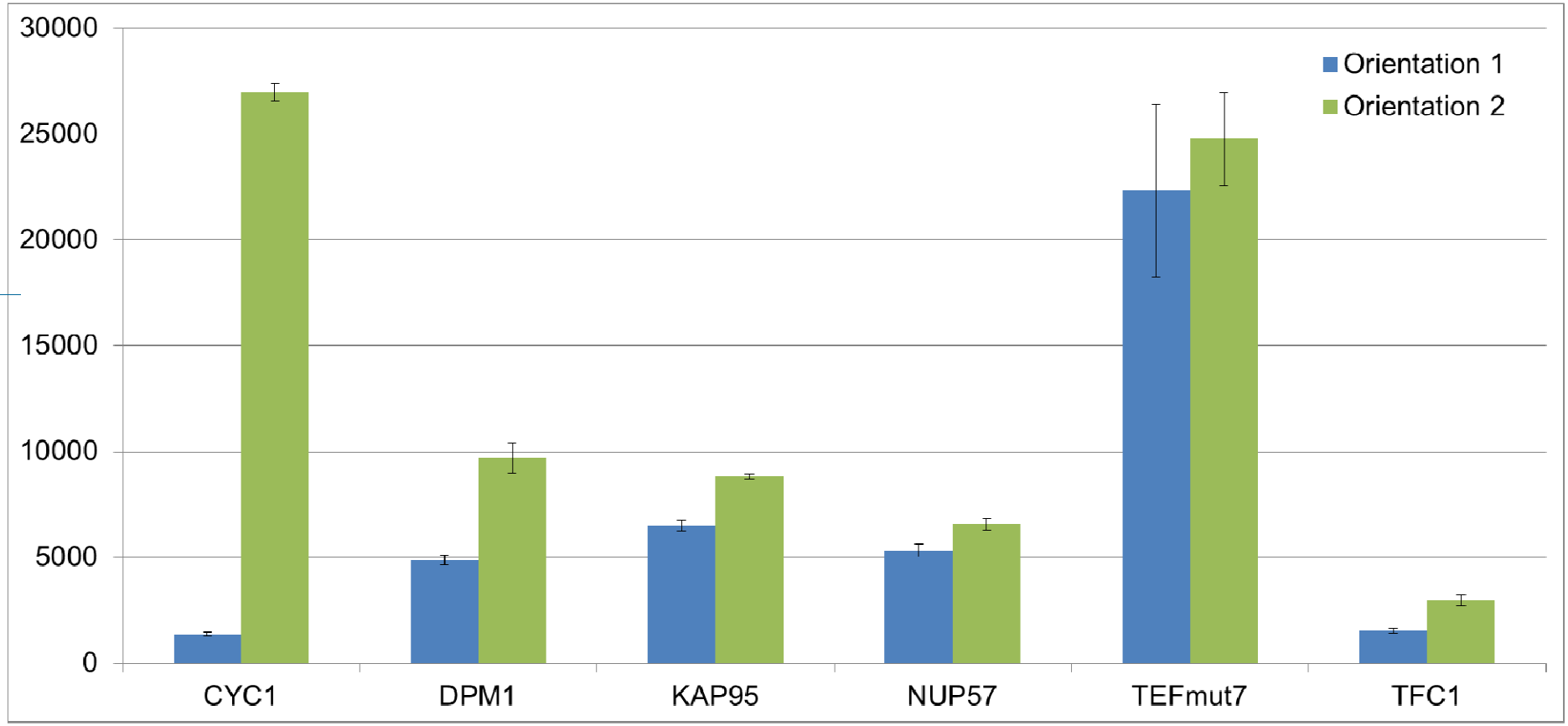
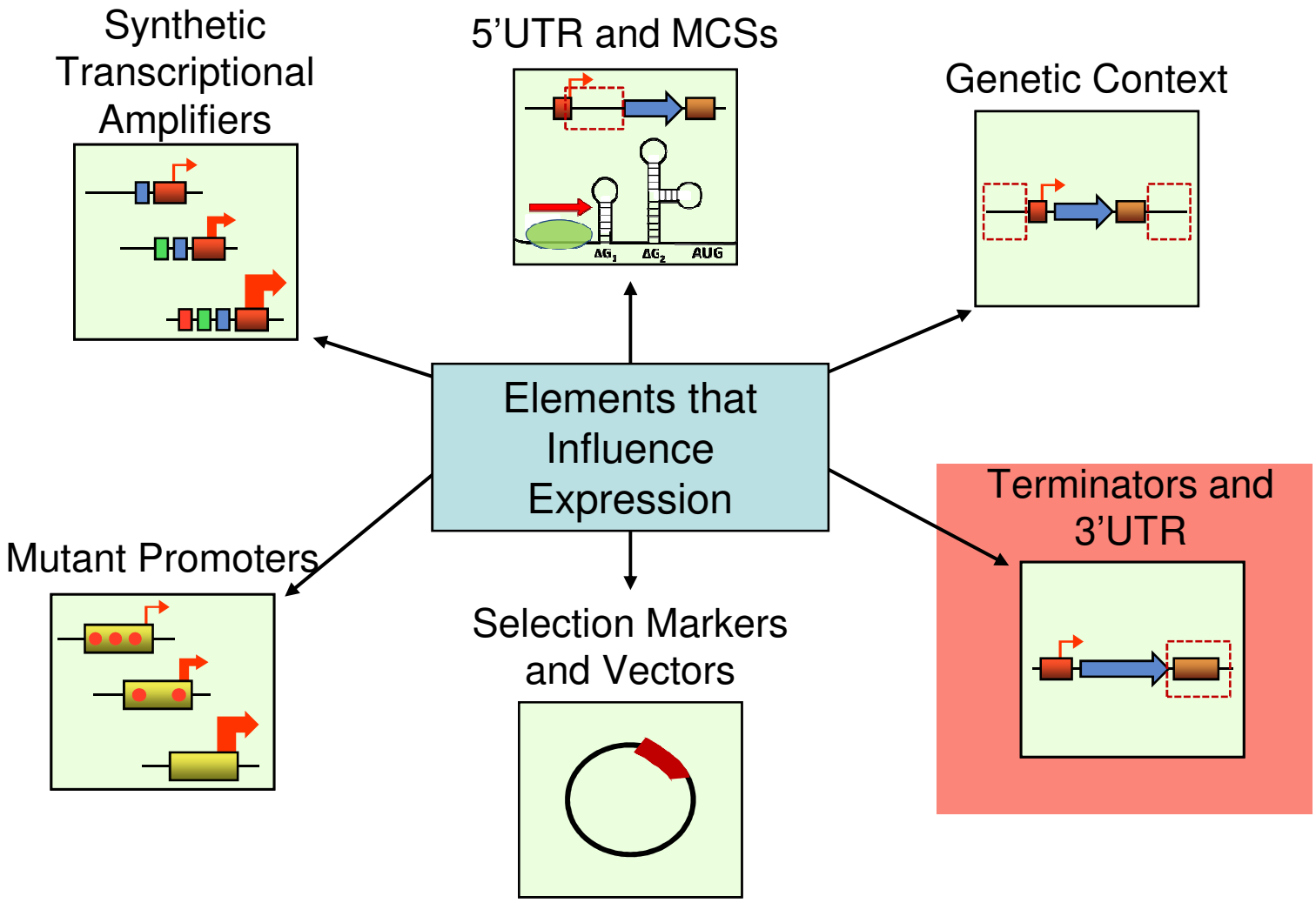


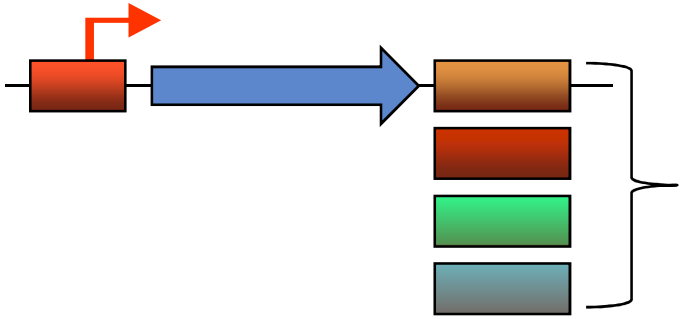
Genetic Context

- Results highlight importance of genetic context of synthetic parts
  - Plasmids vs. genomic integration site
- Highlight need for insulating elements

Synthetic control of transcription occurs at many levels

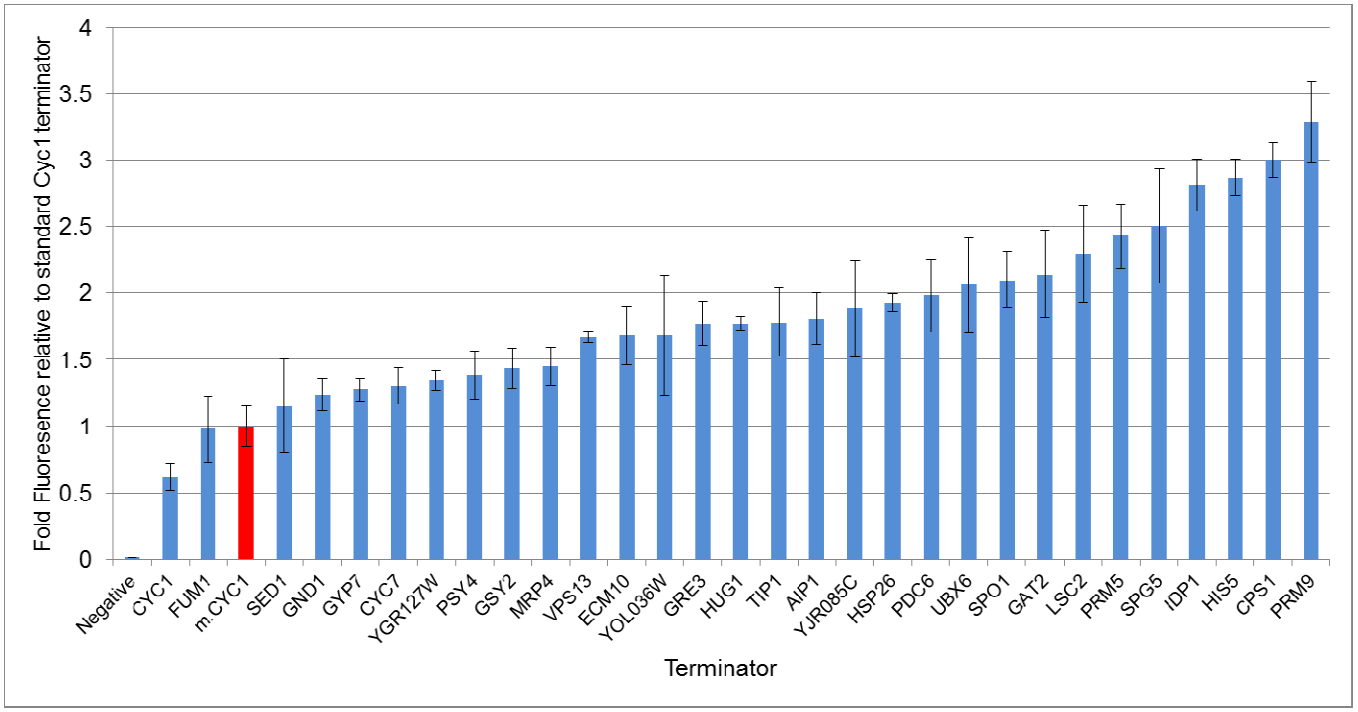


Terminator choice can influence performance



Test impact of changing terminator region

>5 fold difference in expression output by changing terminator



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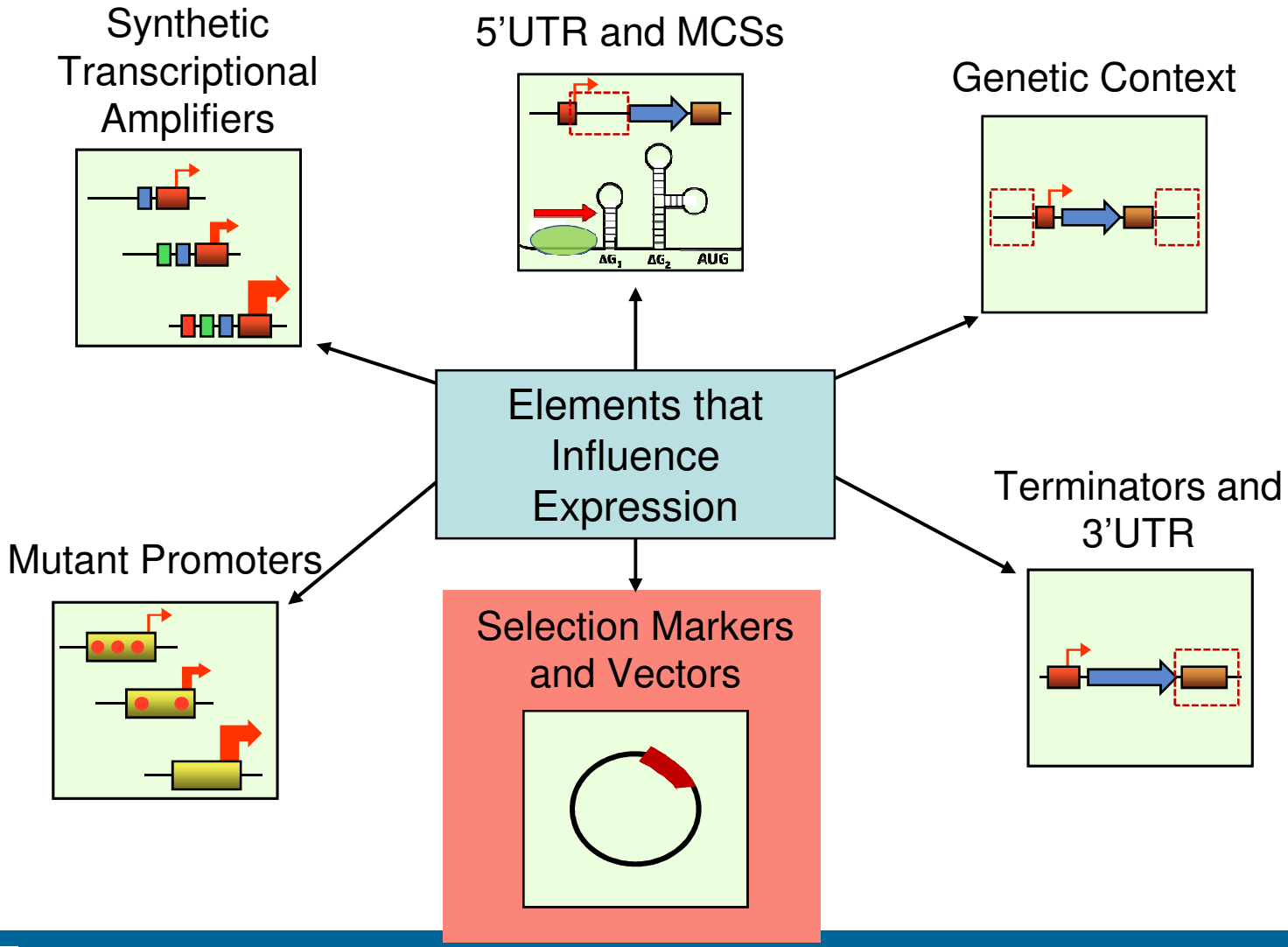

Final Thoughts

- Synthetic control elements are a critical tool for implementing M.E. strategy
- “Promoters” cannot be thought of as singular, isolated elements
- Currently require slightly more range, but significantly more sequence diversity
- Starting to understanding fundamental design principles for this critical components
- These elements are greatly enhancing our capacity to metabolically engineer pathways

Acknowledgements

Students

Graduate	*Undergraduate*
John Blazeck	Vaibhav Agarwala
Joseph Cheng	Heming Bai
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John Leavitt	Rishi Garg
Sun-mi Lee	Rachelle Gerstner
Leqian Liu	Akash Gupta
Heidi Redden	Daniel Huang
Eric Young	Taylor Jellison
	Ashty Karim
	Do Soon Kim
	Peter Otoupal
	Ashley Poucher
	Annie Pan
	Heidi Redden
	Ben Reed
	Lindsey Rey
	Andrea Zuzack